Reference: *Biol. Bull.* **187**: 76–83. (August, 1994)

Osmoregulation in *Dreissena polymorpha*: the Importance of Na, Cl, K, and Particularly Mg

THOMAS H. DIETZ, DIONDI LESSARD, HAROLD SILVERMAN, AND JOHN W. LYNN

Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract. Zebra mussels (*Dreissena polymorpha*) are unusual in that they cannot survive in Mg-deficient water. Analysis of blood samples from mussels obtained in the field indicated a Mg concentration of 1.5–2.0 m*M* immediately after animal collection. However, Mg concentration in the blood decreased rapidly when the mussels were transferred to Mg-free artificial pondwater (PW); the $t_{1/2}$ was 24 h. Blood Mg decreased to the limits of detection within 2 weeks, and the time to 50% mortality was about 17 days in Mg-free PW. When Mg-depleted specimens of *D. polymorpha* were returned to PW containing Mg, the net flux was 3 μmol Mg (g dry tissue · h)$^{-1}$, and blood Mg concentration was restored within a day to 0.4–0.6 m*M*. Mussels depleted of Mg did not survive beyond 51 days. When mussels were acclimated to K-free pondwater (containing Mg), their osmoregulatory ability was impaired, and the total solute of the blood dropped from 30–36 to 21–24 mosm, with blood Na and Cl concentrations declining 30–50%. This ion-depleted condition was reversed within 45 h upon return of K to the pondwater bathing medium. *D. polymorpha* individuals were unable to survive beyond 5 days in deionized water and required minimal concentrations of Na, Cl, K, and Mg for prolonged storage (>51 days) under laboratory conditions. Mussels survived Ca-deficient solutions for more than 51 days, presumably because they were able to mobilize Ca from internal stores (shell) to maintain blood calcium at 1 m*M*.

Introduction

In previous studies, we demonstrated that the tolerance of *Dreissena polymorpha* (zebra mussel) to elevated ion concentrations was notably lower than that of other freshwater bivalves (Horohov *et al.*, 1992). Krogh (1939) reported that although 3 days of salt depletion were required to stimulate Cl uptake in *Dreissena*, it took weeks to stimulate ion uptake in unionids. More recent studies have demonstrated that exposure of *D. polymorpha* to deionized water (DW) causes small mussels to begin dying within days, and all die within 30 days (Nichols, 1993; Ram and Walker, 1993). This inability to tolerate "pure" water is unusual for a freshwater animal; other freshwater bivalves (corbiculids, mytilids, unionids) will survive for months in DW with no mortality (Krogh, 1939; McCorkle and Dietz, 1980; Scheide and Dietz, 1982; Deaton *et al.*, 1989). The addition of either 0.5 m*M* NaCl or 0.5 m*M* $MgSO_4$ promotes short-term survival of zebra mussels compared to animals maintained in DW, and it was suggested that the absence of solute (<1 mosm) was more critical than any specific ion requirement (Ram and Walker, 1993).

Although magnesium transport has been studied extensively in many cells and tissues (Flatman, 1991; Beyenbach *et al.*, 1993) and recently in the freshwater carp (van der Velden *et al.*, 1991), the importance of Mg in the physiology of freshwater bivalves has not been reported. We have observed that survival of freshwater dreissenids in Mg-free pondwater (PW) is density dependent. For example, zebra mussels isolated in individual containers containing Mg-free PW or maintained in small groups (fewer than 20 animals in a liter of Mg-free PW) showed substantial mortality when the experiment lasted longer than 2–3 weeks (pers. obs.). Yet *D. polymorpha* maintained at high density (>100 mussels/l) in the same Mg-free PW suffered virtually no mortality over the same interval. Only when we stored animals longer than 2 months, unfed, in Mg-free PW did we observe some mortality in these mussels (Horohov *et al.*, 1992). Recently, an analysis of the chemical composition of lakes showed that zebra mussels were absent from lakes having low

Received 28 February 1994; accepted 1 June 1994.

concentrations of Ca (<0.5 m*M*); these lakes are also low in Mg (<0.1 m*M*) (Ramcharan *et al.*, 1992).

This is the first report demonstrating that a freshwater bivalve, *D. polymorpha*, has an absolute requirement for external Mg for survival. In addition, K, an ion identified as toxic to freshwater bivalves in concentrations above 0.3–0.5 m*M* (McMahon, 1991), is required in low concentrations for zebra mussels to maintain Na and Cl balance.

Materials and Methods

Animals

Specimens of *Dreissena polymorpha* were collected from Lake Erie at the mouth of the Raisin River in Michigan and used for most of this study. In addition, some mussels were collected from the Mississippi River near Baton Rouge, as noted in the text. Animals were acclimated to an artificial pondwater (PW) normally used for freshwater bivalves (0.5 m*M* NaCl, 0.2 m*M* $NaHCO_3$, 0.05 m*M* KCl, 0.4 m*M* $CaCl_2$) but containing 0.2 m*M* magnesium sulfate. The amount of Mg present in fresh water is extremely variable, but many rivers and other bodies of water tend to have a Ca:Mg ratio of 2:1, and we have selected this relationship for the artificial PW used for zebra mussels (Withers, 1992). Mussels were stored unfed in aerated PW at 22 ± 2°C and survived for months at this temperature with little mortality. For longer maintenance, mussels were held in PW at 16 ± 1°C and subsequently transferred to room temperature 5–7 days before use. We selected larger specimens for study (1.5–3 cm length) with dry tissue masses (excluding the shell) of 15–80 mg.

To prevent contamination of local water systems with mussels or veliger larvae, mussel tissue was excised from the shell and dried to constant weight at 95°C before being discarded. In addition, all acclimation water and animal containers were treated with 1% chlorine bleach for 24 h before being discarded.

Blood analysis

Blood was collected by heart puncture and centrifuged (14,000 $g \cdot$ min) before use (Fyhn and Costlow, 1975). We routinely collected blood volumes (>150 μl) equal to 10–20% of animal wet weight. Several animals were dissected to determine landmarks that would ensure pericardial puncture and prevent the collection of stomach fluid. Evidence of contamination included visible particulate material, the green color of algae, and premature freezing of the blood sample in the osmometer.

Total solute in the blood was determined by freezing point depression. Sodium and potassium concentrations were determined by emission flame photometry. Calcium and magnesium were diluted in $LaCl_2$ and assayed by atomic absorption spectroscopy. Chloride was determined by electrometric titration. Although the bicarbonate concentrations were not routinely measured, we have estimated the concentration previously (see Horohov *et al.*, 1992). These analytical methods ensured complete ionization and maximum estimates of element concentrations. The difference between the total solute and the sum of the measured ions was identified as "other" and is assumed to be mostly bicarbonate.

Ion net flux

Previously described methods (Graves and Dietz, 1982) were used to calculate net ion flux (J_n) from the change in ion concentration in the bathing medium. For flux studies, we chose animals that were attached (to the substrate or to each other) by byssal threads, and detached them by cutting the threads to avoid injuring the animals. The mussels were rinsed in DW for about 30 min and transferred to small beakers containing the appropriate bathing solution. Bath samples were collected at specific intervals, and J_n was calculated from the change in ion concentration in the bathing medium. Net ion flux was expressed as μmol (g dry tissue · h)$^{-1}$.

Survival in ion deficient solutions

To characterize the sensitivity of *D. polymorpha* to ions in the bathing medium, we transferred groups of 20 animals into either 1 l of DW or 1-l solutions of the 31 permutations of the salts present in PW (see Table III). During the study the shells of six animals were accidentally damaged, so these specimens were eliminated from the study. The solutions, covered to minimize evaporation, were continuously aerated and replaced on alternate days. The animals were examined regularly: gaping animals were touched with forceps to stimulate valve closure; unresponsive specimens were touched in the area of the siphons. If there were no valve or tissue responses, the animal was considered dead. Dead animals were removed from the container, the time noted, and the water changed. The time required to reach 50% mortality (LT_{50}) in each solution was derived from the probit model (Finney, 1971). Mortality rates were curvilinear, and the cumulative mortality was assigned a probability (probit) ranging from 0 to 1 at each point when an animal died. The LT_{50} was calculated from the linear regression of the logarithm of time and cumulative mortality:

$$\text{probit (\%dead)} = A + B \text{ (log time, } h)$$

The time of death of the last animal in the test solution was reported as the time to 100% sample mortality (SM_{100}). Animals in 10 of the solutions, including the PW controls, had little or no mortality, and the experiment

Table I

Blood ion composition in Dreissena polymorpha *collected from the Mississippi River and transferred to Mg-free artificial pondwater (PW)*

Days in Mg-free PW	mosm (solute)	Concentration (m*M*)					
		Na	Ca	Mg*	K	Cl	Other
0	42 ± 0 (8)	17.4 ± 0.1 (8)	4.9 ± 0.2[a] (8)	1.50 ± 0.20[a] (8)	0.4 ± 0.0 (8)	16.9 ± 0.5[b] (8)	1.0 ± 0.4[b] (8)
2	42 ± 2 (4)	17.7 ± 0.4 (6)	3.6 ± 0.1[b] (6)	0.33 ± 0.02[b] (6)	0.5 ± 0.0 (6)	19.3 ± 0.4[a] (6)	1.5 ± 0.8[b] (4)
9	41 ± 1 (7)	17.2 ± 0.2 (7)	3.3 ± 0.3[b] (7)	0.13 ± 0.01[c] (7)	0.4 ± 0.0 (7)	14.3 ± 0.5[c] (7)	5.7 ± 0.4[a] (7)
15	42 ± 1 (8)	17.8 ± 0.6 (8)	5.4 ± 0.2[a] (8)	0.07 ± 0.01[d] (8)	0.5 ± 0.0 (8)	14.0 ± 0.6[c] (8)	4.1 ± 0.6[a] (8)

Means ± SEM with number of animals in parentheses. Total solute was rounded to 2 significant figures. "Other" represents the difference between the total solute and the sum of the measured ions. Values with different letters are significantly different (a > b > c > d) according to the Tukey-Kramer Multiple Comparison test ($P < 0.05$).

* Data were transformed by natural logarithm for analysis.

was terminated when only these groups of animals remained. Blood was collected from seven survivors from each of these 10 solutions. The remaining survivors from the K-deficient solutions were transferred into PW. The animals were allowed 45 h in which to replete missing ions, and blood samples were then collected. All animals survived the transfer to PW.

Statistical analysis

Data are expressed as means ± 1 standard error; the number of animals is in parentheses. To analyze the effects of PW ions on mussel blood composition, the data were analyzed by ANOVA, and if significant differences between means were found, we used the Tukey's Studentized Range test or, if the sample sizes were unequal, the Tukey-Kramer modification. Differences were considered significant if $P < 0.05$. Ion concentrations that were significantly different in the different treatments were assigned a letter, with "a" denoting the highest values. Bartlett's test for homogeneity of variance was used to determine whether there were significant differences in the standard deviations among groups, and the appropriate data sets were transformed by natural logarithm.

Results

Blood composition

The blood ion concentration in *D. polymorpha* was rather variable, depending on the source of the animals. Animals sampled at the time of their collection from the mouth of the Raisin River in Michigan (20°C) had a blood total solute of 50 ± 1 (8) mosm and a Mg concentration of 2.06 ± 0.12 m*M*. Zebra mussels collected from the Mississippi River near Baton Rouge (25°C) had significantly lower total solute of 42 ± 0 (6) mosm ($P < 0.01$) and [Mg] of 1.50 ± 0.20 m*M* ($P < 0.02$). The lower Mississippi River approximates what Withers (1992) classified as an "average" river in terms of ion concentrations (in m*M*: 0.9 Na, 0.8 Ca, 0.4 Mg, 0.17 K, 0.55 Cl, 0.5 SO_4, 2.7 unidentified). The composition of the blood from animals collected in Michigan was similar to, but significantly higher in total solute than, that found in freshly collected animals from the Mississippi River. Water temperature, however, differed by 5°C between the two locations. After 1–2 weeks acclimation to artificial PW, the blood composition of animals from Michigan and Louisiana tended to become indistinguishable; total solute of the blood stabilized at about 40–45 mosm, with Mg usually about 0.4–0.6 m*M*.

Mg depletion and repletion

Transferring zebra mussels directly from the Mississippi River into Mg-free PW resulted in a significant exponential decline ($r = 0.93$, $n = 27$) in blood [Mg] with a $t_{1/2}$ of about 1 day (Table I):

$$\text{Blood [Mg]} = 1.5\ \text{m}M\ e^{-(0.73 \cdot \text{days of Mg depletion})}$$

The total solute, [Na], and [K] did not change over a 2-week interval, but [Cl] decreased significantly and "other" (presumably HCO_3) was elevated (see Horohov *et al.*, 1992). Blood [Ca] initially declined during Mg-free PW acclimation but increased significantly with severe Mg depletion. Similar results have been obtained with mussels from Lake Erie (data not shown).

Transferring Mg-depleted zebra mussels to PW containing variable concentrations of Mg resulted in a rapid uptake of Mg that appeared to be maximal when the external concentration was between 0.1 and 0.2 m*M* Mg

(Table II). Following the 6-h net flux study, the animals were left in the solution overnight (17 h). Subsequent sampling showed that the Mg concentration in the blood of these mussels had been restored to levels comparable to those in animals continually maintained in PW (compare Tables II and IV). Also, the total solute in these animals was significantly elevated, primarily because of a modest elevation in the concentrations of both Na and Cl. The sum of the change in Na and Cl concentration accounted for 10 of the 13 mosm total solute increase. The negative value for "other" in the Mg-depleted animals was not significantly different from zero. However, we occasionally calculated the sum of the measured ions as slightly exceeding the total solute for some animals; thus our Ca values may be inflated due to complete Ca ionization (even of bound Ca) produced by the analytic procedures. A separate group of 20 zebra mussels acclimated to PW containing 0.05 m*M* $MgSO_4$ survived more than 60 days with only one death.

Selective ion depletion

Several salts constitute the artificial pondwater, and we examined the influence of each on the survival of zebra mussels. Mussels were maintained in solutions to which salts were added singly or in combination at the concentration present in PW. The LT_{50} and SM_{100} were determined, and the total numbers of animals that died are presented in Table III. All solutions deficient in Na, Cl, and Mg were lethal, and a minimal concentration of these ions was required for a 51-day survival. Mussels in DW or solutions deficient in Na did not survive a week. Survival was extended (LT_{50} = 2 weeks) in solutions containing only Na salts, but if Ca was present the LT_{50} was reduced. Magnesium by itself and in all combinations of salts with [Na] less than 0.2 m*M* did not promote survival beyond an LT_{50} of about 30 days. But the combination of 0.2 m*M* $NaHCO_3$, 0.05 m*M* KCl, and 0.2 m*M* $MgSO_4$ allowed the animals to survive for 51 days and, based on the apparently normal (51-day) blood composition, probably indefinitely (Table IV). A solution with $CaCl_2$ and $MgSO_4$ as the only solutes was among the more toxic combinations; but the addition of Na extended survival substantially. These data suggest that both the Ca:Mg ratio and the level of Na are critical to zebra mussel survival.

Prolonged storage of *D. polymorpha* in PW without feeding reduced total solute in the blood in PW controls to 30–35 mosm. Ion concentrations in blood samples taken from animals that survived the 51-day differential ion combination study are shown in Table IV. Magnesium, Na, and Cl were required for the 51-day survival, but if K was absent the measured blood ion concentrations were significantly lower than those found in PW. Most of the animals in K-deficient media survived and were attached by byssal threads, but all had significantly reduced total solute and significantly lower blood K, Na, and Cl concentrations—perhaps reduced to near the survival limit for these mussels. Animals in Ca-deficient solutions had significantly depressed blood [Ca] but elevated [Mg], suggesting a reciprocal relationship between these two ions.

Repletion of blood ions in K-depleted mussels

When the K-depleted mussels were returned to complete PW for 45 h of recovery, the missing ions were rapidly reaccumulated and the ion concentrations were restored to near normal (Table V, compare with Table IV).

Table II

Net Mg flux (over 6 h) and blood ion composition in Mg-depleted Dreissena polymorpha *17 h after reaccumulating ions from pondwater (PW) containing Mg*

Treatment	$^{Mg}J_{net}$ μmol $(g \cdot h)^{-1}$	mosm (Total)	Concentration (m*M*)					
			Na	Ca	Mg*	K	Cl	Other
PW (0 m*M* Mg)	0 ± 0^{b}	37 ± 3^{b}	15.0 ± 2.0	5.2 ± 0.4	0.02 ± 0.02^{c}	0.4 ± 0.1	17.9 ± 1.4	−1.0 ± 0.9
	(5)	(5)	(4)	(5)	(5)	(4)	(4)	(4)
PW (0.05 m*M* Mg)	1.2 ± 0.2^{b}	45 ± 2^{b}	19.0 ± 1.0	3.8 ± 0.7	$0.15 \pm 0.03^{b,c}$	0.4 ± 0.0	20.6 ± 1.2	1.5 ± 1.0
	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
PW (0.1 m*M* Mg)	3.8 ± 0.8^{a}	48 ± 3^{a}	17.2 ± 3.0	3.9 ± 0.7	0.20 ± 0.03^{b}	0.3 ± 0.1	21.2 ± 1.8	2.3 ± 1.4
	(5)	(5)	(3)	(5)	(5)	(3)	(4)	(3)
PW (0.2 m*M* Mg)	3.2 ± 0.4^{a}	50 ± 2^{a}	20.3 ± 1.1	3.6 ± 0.4	0.50 ± 0.9^{a}	0.4 ± 0.0	22.4 ± 1.1	2.2 ± 1.1
	(5)	(5)	(5)	(5)	(5)	(5)	(4)	(4)

Means ± SEM with number of samples in parentheses. Total solute was rounded to 2 significant figures. Values with different letters are significantly different (a > b > c) with the Tukey-Kramer Multiple Comparison test ($P < 0.05$).

* Data were transformed by natural logarithm for analysis.

Table III

Lethal time for 50% (LT) and 100% sample mortality (SM) of zebra mussels in solutions of single salts or mixtures of salts at pondwater (PW) ion concentrations

Ions	Days		
	LT_{50}	SM_{100}	*n* died
Distilled water	3.9	4.9	20
$MgSO_4$	1.5	3.9	20
KCl	1.7	3.9	20
$CaCl_2$	1.8	3.9	20
$NaHCO_3$	14.1	22.9	19*
NaCl	15.2	35.4	20
$CaCl_2$, $MgSO_4$	1.7	4.0	20
KCl, $CaCl_2$	2.5	3.9	20
KCl, $MgSO_4$	4.2	6.9	20
$NaHCO_3$, $CaCl_2$	6.9	12.0	20
NaCl, $CaCl_2$	12.4	37.3	20
NaCl, KCl	21.2	43.4	20
$NaHCO_3$, KCl	26.4	44.3	17*
$NaHCO_3$, NaCl	28.4	50.3	20
$NaHCO_3$, $MgSO_4$	29.8	48.8	20
NaCl, $MgSO_4$	—	>51	0
KCl, $CaCl_2$, $MgSO_4$	1.4	4.0	20
KCl, $NaHCO_3$, $CaCl_2$	13.1	17.9	20
NaCl, $NaHCO_3$, $CaCl_2$	14.9	23.8	20
NaCl, KCl, $CaCl_2$	16.9	44.8	20
NaCl, KCl, $NaHCO_3$	28.6	44.3	20
$NaHCO_3$, $CaCl_2$, $MgSO_4$	29.6	44.3	20
$NaHCO_3$, KCl, $MgSO_4$	—	>51	1
NaCl, KCl, $MgSO_4$	—	>51	1
NaCl, $NaHCO_3$, $MgSO_4$	—	>51	0
NaCl, $CaCl_2$, $MgSO_4$	—	>51	1
NaCl, KCl, $NaHCO_3$, $CaCl_2$ (Mg-free PW)	16.9	31.3	20
NaCl, KCl, $CaCl_2$, $MgSO_4$	—	>51	2
NaCl, KCl, $NaHCO_3$, $MgSO_4$	—	>51	3
KCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$	—	>51	0
NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$	—	>51	2
NaCl, KCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$ (PW)	—	>51	1

PW = 0.5 m*M* NaCl, 0.05 m*M* KCl, 0.2 m*M* $NaHCO_3$, 0.4 m*M* $CaCl_2$, 0.2 m*M* $MgSO_4$.

* Less than 20 because some animals were accidentally damaged and were removed from the test.

The K balance was critical to the ability of the zebra mussel to maintain Na and Cl homeostasis. With the restoration of Ca in the PW, the blood Ca:Mg ratio returned to normal (Ca > Mg) as a result of both an elevation in the blood [Ca] and a decline in [Mg]. We have repeated the ion tolerance study using Mg-depleted mussels acclimated to PW. These animals were exposed to combinations of PW salts that excluded Mg. We observed a similar qualitative pattern of survival times, and no animal survived beyond 52 days (data not shown). The animals with the longest SM_{100} (52 days) were in the NaCl, KCl solution and the NaCl, KCl, and $NaHCO_3$ solution (LT_{50} 21 and 31 days, respectively), whereas mussels in deionized water had an LT_{50} of 2.3 days.

Discussion

Unionids and corbiculids can be maintained in the laboratory in Mg-free pondwater or deionized water for months. These two groups invaded fresh water separately, with the corbiculids being more recent (Pleistocene), and there are significant differences in their physiologic processes (Dietz, 1979; McMahon, 1991). The dreissenids represent a third independent (Pleistocene) invasion of fresh water (McMahon, 1991), and *Dreissena polymorpha* exhibits remarkably complex ion requirements not observed in other freshwater bivalves. Zebra mussels lose 50% of their blood [Mg] in one day when placed in Mg-deficient solutions. Apparently these animals are similar to fish in that reabsorption of Mg from the ultrafiltrate entering the kidney is limited; thus, urinary loss of Mg is substantial (Beyenbach *et al.*, 1993). In addition, the mantle or gill epithelia of zebra mussel may have a high passive permeability to Mg, contributing to the loss of this ion. Although the initial loss of Mg is remarkably rapid, a mechanism with a limited ability to reduce the rate of loss of Mg appears to become operational with prolonged depletion. With a $t_{1/2}$ of 1 day for the loss of Mg from the blood, the Mg should be unmeasurable within 7 half-lives, yet nearly 2 weeks were required for blood [Mg] to drop to zero (i.e., below detection limits).

Although the *D. polymorpha* individuals we tested experienced a high rate of Mg loss, their transport system rapidly took up Mg from PW (0.1–0.2 m*M* Mg). This transport system restored the blood Mg concentration in less than a day when Mg-depleted mussels were returned to solutions containing Mg. Moreover, they survived for more than 60 days with little mortality in PW containing 0.05 m*M* $MgSO_4$. The ^{28}Mg isotope is not currently available in the United States, so we have not measured the unidirectional flux. In carp, Mg is transported into the animal from fresh water by epithelial Mg transport, but intestinal uptake from dietary sources is required to maintain Mg balance (van der Velden *et al.*, 1991). Although dietary Mg may allow *D. polymorpha* to maintain blood [Mg] above 1 m*M*, unfed PW-acclimated mussels had blood [Mg] between 0.4 and 0.6 m*M*. In addition, all zebra mussels in Ca-deficient media containing 0.2 m*M* $MgSO_4$ had a blood [Mg] above 1 m*M*.

When minimal concentrations of Mg, Na, Cl, and K were in the bathing solution, *D. polymorpha* had blood solute levels near the low end of the normal range (>30 mosm) for bivalves. However, members of this species could not survive salt depletion in deionized water or selective depletion of any of the four critical ions for more than a short period (this study; Nichols, 1993; Ram and

Table IV

Blood ion concentration in Dreissena polymorpha *surviving 51 days in pondwater (PW) and solutions deficient in salts present in artificial PW*

Incubation medium	mosm (Total)	Concentration (m*M*)					
		Na	Ca	Mg	K	Cl	Other
PW, day 0	38 ± 1^{a}	15.6 ± 0.7^{a}	3.3 ± 0.1^{a}	0.4 ± 0.0^{c}	0.4 ± 0.0^{b}	16.0 ± 0.5^{a}	2.3 ± 0.5
PW, day 51	$33 \pm 1^{a,b,c}$	$13.2 \pm 0.2^{a,b,c}$	$1.8 \pm 0.1^{c,d}$	0.6 ± 0.0^{c}	$0.5 \pm 0.0^{a,b}$	$14.3 \pm 0.8^{a,b}$	2.2 ± 0.4
NaCl, KCl, $NaHCO_3$, $MgSO_4$	$36 \pm 1^{a,b}$	16.1 ± 1.0^{a}	1.0 ± 0.2^{e}	$1.3 \pm 0.1^{a,b}$	0.6 ± 0.0^{a}	$14.2 \pm 0.7^{a,b}$	3.1 ± 1.0
NaCl, KCl, $CaCl_2$, $MgSO_4$	$35 \pm 2^{a,b,c}$	15.7 ± 1.0^{a}	$1.7 \pm 0.2^{c,d}$	0.5 ± 0.0^{c}	0.5 ± 0.0^{a}	$15.3 \pm 0.9^{a,b}$	1.6 ± 0.3
$NaHCO_3$, KCl, $CaCl_2$, $MgSO_4$	$31 \pm 1^{b,c}$	$11.1 \pm 0.6^{c,d,e}$	$2.4 \pm 0.2^{b,c}$	0.7 ± 0.1^{c}	$0.5 \pm 0.0^{a,b}$	$14.1 \pm 0.4^{a,b}$	2.5 ± 0.6
NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$	21 ± 1^{d}	6.3 ± 0.9^{f}	2.5 ± 0.1^{b}	0.6 ± 0.1^{c}	0.1 ± 0.0^{c}	7.7 ± 0.9^{d}	3.4 ± 0.8
NaCl, KCl, $MgSO_4$	$34 \pm 1^{a,b,c}$	$14.4 \pm 0.4^{a,b}$	1.0 ± 0.1^{e}	1.2 ± 0.1^{b}	0.5 ± 0.0^{a}	$14.3 \pm 0.6^{a,b}$	2.8 ± 0.5
$NaHCO_3$, KCl, $MgSO_4$	30 ± 1^{c}	$12.0 \pm 0.8^{b,c,d}$	$1.2 \pm 0.1^{d,e}$	$1.5 \pm 0.1^{a,b}$	$0.5 \pm 0.0^{a,b}$	$12.4 \pm 0.5^{b,c}$	2.6 ± 0.3
NaCl, $CaCl_2$, $MgSO_4$	23 ± 1^{d}	$8.4 \pm 0.5^{e,f}$	$2.3 \pm 0.2^{b,c}$	0.6 ± 0.0^{c}	0.1 ± 0.0^{c}	$9.7 \pm 0.6^{c,d}$	1.8 ± 0.4
NaCl, $NaHCO_3$, $MgSO_4$	24 ± 2^{d}	$8.3 \pm 0.7^{e,f}$	$1.2 \pm 0.1^{d,e}$	1.8 ± 0.1^{a}	0.1 ± 0.0^{c}	$9.1 \pm 1.0^{c,d}$	3.2 ± 0.5
NaCl, $MgSO_4$	24 ± 1^{d}	$8.9 \pm 0.5^{d,e,f}$	$1.3 \pm 0.2^{d,e}$	1.2 ± 0.2^{b}	0.1 ± 0.0^{c}	8.6 ± 0.8^{d}	3.5 ± 0.7

Means ± SEM ($n = 7$). Total solute was rounded to 2 significant figures. PW = 0.5 m*M* NaCl, 0.05 m*M* KCl, 0.2 m*M* $NaHCO_3$, 0.4 m*M* $CaCl_2$, 0.2 m*M* $MgSO_4$. Means with different letters are significantly different (a > b > c > d > e > f) according to the Tukey Studentized Range test, $P < 0.05$.

Walker, 1993; Vinogradov *et al.*, 1993). The zebra mussel requirement for specific ions and "threshold" ion concentrations in the bathing medium is in contrast with the tolerance shown by other freshwater bivalves for prolonged salt depletion or selective ion depletion (Krogh, 1939; Murphy and Dietz, 1976; McCorkle and Dietz, 1980; Scheide and Dietz, 1982; Deaton *et al.*, 1989). The PW used in our laboratory for these earlier bivalve studies was Mg-free. The bivalves used in these previous studies have mechanisms that reduce the loss of ions by decreasing ion efflux as well as by increasing the influx of the ions independently of exogenous Mg.

Shell growth in zebra mussels and other freshwater bivalves requires Ca in the environment. Calcium mobilization from the reservoir in the shell allows bivalves to survive in Ca-deficient solutions. The endogenous sources of calcium were sufficient to maintain zebra mussel blood [Ca] at about 1 m*M* in Ca-free solutions. In contrast, ion depletion or other physiological challenges result in elevated blood [Ca] in other bivalves (Scheide and Dietz, 1982; Deaton *et al.*, 1989; Byrne *et al.*, 1991). However, with the mobilization of Ca and presumably CO_3 from the shell, limited amounts of HCO_3 ("other," in data tables) were retained in the blood of *D. polymorpha.*

The rather low blood bicarbonate concentration found in zebra mussels is similar to that in the Canadian unionid *Anodonta grandis simpsoniana* (Byrne and McMahon, 1991). In contrast, HCO_3 is substituted for Cl in the blood of other bivalves (Byrne *et al.*, 1991; Scheide and Dietz, 1982; Deaton *et al.*, 1989). Indeed, blood [HCO_3] of sev-

Table V

Blood ion concentration in Dreissena polymorpha *allowed to reaccumulate ions from pondwater (PW) for 45 h after surviving 51 days in PW or solutions containing Mg but deficient in K and one or more other ions*

Original incubation medium	n	mosm (Total)	Concentration (m*M*)					
			Na	Ca	Mg	K	Cl	Other
PW, day 0	7	$38 \pm 1^{a,b}$	$15.6 \pm 0.7^{a,b}$	3.3 ± 0.1^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0	16.0 ± 0.5	2.4 ± 0.5
PW, day 51	7	33 ± 1^{b}	13.2 ± 0.2^{b}	$1.8 \pm 0.1^{b,c}$	0.6 ± 0.0^{b}	0.5 ± 0.0	14.3 ± 0.8	2.2 ± 0.4
NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$	4	$34 \pm 1^{a,b}$	$14.8 \pm 0.5^{a,b}$	$1.6 \pm 0.1^{b,c}$	$0.6 \pm 0.0^{a,b}$	0.4 ± 0.0	12.7 ± 1.6	4.4 ± 1.3
NaCl, $CaCl_2$, $MgSO_4$	5	38 ± 1^{a}	16.8 ± 0.5^{a}	2.3 ± 0.4^{b}	$0.7 \pm 0.0^{a,b}$	0.4 ± 0.0	13.2 ± 1.5	4.7 ± 0.6
NaCl, $NaHCO_3$, $MgSO_4$	4	$34 \pm 2^{a,b}$	$14.4 \pm 1.7^{a,b}$	1.4 ± 0.1^{c}	0.9 ± 0.2^{a}	0.4 ± 0.0	15.0 ± 1.0	2.0 ± 0.3
NaCl, $MgSO_4$	5	$36 \pm 1^{a,b}$	$14.6 \pm 0.7^{a,b}$	$1.9 \pm 0.2^{b,c}$	0.9 ± 0.1^{a}	0.4 ± 0.1	14.8 ± 0.8	3.4 ± 1.0

Means ± SEM, total solute was rounded to 2 significant figures. PW = 0.5 m*M* NaCl, 0.05 m*M* KCl, 0.2 m*M* $NaHCO_3$, 0.4 m*M* $CaCl_2$, 0.2 m*M* $MgSO_4$. The PW controls are the same values listed in Table IV. Values with different letters are significantly different (a > b > c) according to Tukey's Studentized Range test, $P < 0.05$.

eral PW-acclimated unionids is equal to or exceeds [Cl] (Dietz, 1979). One of the better single salts for extending survival of zebra mussels (LT_{50} = 2 weeks) was $NaHCO_3$, and the survival was doubled (LT_{50} = 4 weeks) with the addition of any ion but Ca.

Half of the animals exposed to calcium-containing solutions without Mg did not survive 2.5 weeks (maximum LT_{50} < 17 days). Apparently the presence of exogenous and endogenous Ca, in the absence of exogenous Mg, resulted in an unfavorable Ca:Mg balance, leading to death. We do not know which functions critical for survival are impaired by Mg deficiency, but candidates range from disruption of epithelial cell junctions to elevation of membrane ion permeability (both factors aggravating ion losses), or Ca interference with Mg-requiring enzymes. Deaton and Greenberg (1991) noted a good correlation between the osmoregulatory ability of bivalves and their capacity to mobilize calcium. They suggested that the ability to regulate membrane permeability to ions was intimately linked to calcium balance, especially the ability to elevate blood [Ca]. Blood [Ca] in *D. polymorpha* was regulated at relatively low concentrations under the conditions we studied, and our data seem to fit Deaton and Greenberg's hypothesis. Yet the role and importance of blood bicarbonate in bivalve osmoregulation is not being considered in this hypothesis and suggests an area meriting further study.

The two salts promoting the longest survival in the ion permutation experiments were NaCl and $MgSO_4$. It is unlikely, on the basis of the substantial loss of blood solutes observed, that the animals would have survived much beyond the study period (51 days) in this minimal salt mixture.

Blood Na and Cl balance could not be maintained following exposure of zebra mussels to solutions deficient in K. The loss of K from the blood and cells probably disrupted the electrochemical gradient required for Na and Cl transport. The [K] tolerance range of zebra mussels is exceptionally narrow. Potassium concentrations above 0.3–0.5 m*M* in PW are lethal to *D. polymorpha* and other freshwater bivalves, yet, unlike other freshwater mussels, zebra mussels cannot survive without K while being stored unfed in the laboratory (Dietz and Byrne, 1990; Fisher *et al.*, 1991; McMahon, 1991; Horohov *et al.*, 1992; Ram and Walker, 1993; Vinogradov *et al.*, 1993). Fed bivalves may survive a year in the laboratory, in part because of the food supplement, but starvation is not a significant short-term problem, because unfed zebra mussels will survive for over 6 months when maintained in Mg-PW. When zebra mussels are fed, the K content of the algal cells and the culture medium used to raise the algae must be monitored. If, during feeding, the K concentration in the bathing medium is elevated above the tolerated level, the zebra mussels will be killed by the excess K, not by too much food.

The ambient level of calcium is an important predictor of zebra mussel presence and has a positive correlation with population density in a variety of lakes that have been modeled (Ramcharan *et al.*, 1992). In contrast, [Mg] displays no relationship with mussel occurrence or abundance. The minimum amount of Mg present in lakes with sufficient Ca to support a high zebra mussel population is 0.1 m*M*, and with low zebra mussel density the minimum amount of Mg is 0.03 m*M*. The 0.03 m*M* Mg concentration is likely to be near the threshold for zebra mussel survival. Sprung (1987) also noted significantly more abnormal embryonic development when *D. polymorpha* was raised in 0.04 m*M* Mg. But since, in zebra mussels, the Mg requirement seems to be for threshold amounts, there is no correlation with mussel population density over a range of Mg concentrations.

According to the model of Ramcharan *et al.* (1992), the Mississippi River ion concentrations are well above the threshold for the factors contributing to zebra mussel populations of high density. Indeed, we observed that both the adult zebra mussels and some larvae survived the summer of 1993 in the Mississippi River, even though temperatures were above 27°C for nearly 3 months. The predictive models developed by Ramcharan *et al.* (1992) suggest that the Mississippi River can support high densities of zebra mussels.

The unique sensitivity of zebra mussels to environmental ion composition may explain some of the variability that has been reported in the survival and blood ion concentrations. Even though zebra mussels are normally found in water with moderate to high calcium concentrations, they will survive low environmental [Ca] providing the bathing fluid contains Mg in minimal amounts. Although zebra mussels have an absolute requirement for Mg in the water, they can be held for months in nominally Mg-free PW if they are stored in high density (>100/l). The Mg lost by one mussel will accumulate in the medium and be available for uptake by adjacent animals. Even the "weaker" animals suffering the greatest Mg loss would survive for weeks if the composition of the ion mixture in the bathing water were adequate.

D. polymorpha represents one of several independent invasions of fresh water, and these mussels have evolved many physiological processes that are in common with other freshwater bivalves (Dietz, 1979; Deaton *et al.*, 1989; Deaton and Greenberg, 1991; McMahon, 1991; Horohov *et al.*, 1992). However, zebra mussel ion transport rates are among the highest found in freshwater mussels and reflect the recent evolution from brackish-water ancestors (Horohov *et al.*, 1992). For survival in fresh water, the ionic uptake rate must be in balance with ionic loss (renal

and extrarenal). Of the freshwater bivalves studied, zebra mussels are least capable of tolerating ion losses associated with salt-depletion conditions imposed by deionized water. Zebra mussels require minimal concentrations of ions (Mg, Na, K, Cl) in their environment for long-term (>50 days) survival. Thus, zebra mussels are the most stenohaline (both low and high salinity) freshwater bivalves that have been studied. Unlike other freshwater bivalves, *D. polymorpha* apparently has not evolved sufficient mechanisms to allow survival in the most dilute of freshwater environments.

Acknowledgments

We thank Drs. Robert McMahon and Roger Byrne for providing the zebra mussels collected from Lake Erie and for their many suggestions and comments. T. R. LeBlanc provided valuable assistance in collecting mussels from the Mississippi River at DOW Chemical, Plaquemine, LA. We also thank S. J. Nichols for many hours of discussion and the use of the USFWS facilities in Ann Arbor, MI. Julie Cherry and Janice Horohov provided technical assistance. D. L. participated with support from a Howard Hughes Medical Institute undergraduate science initiative grant to LSU. This research was supported, in part, by the LSU Center for Energy Studies grant 91-01-11 continuation and NSF grant DCB90-17461.

Literature Cited

Beyenbach, K. W., C. A. Freire, R. K. Kinne II, and E. Kinne-Saffran. 1993. Epithelial transport of magnesium in the kidney of fish. *Miner. Electrolyte Metab.* **19:** 241–249.

Byrne, R. A., and B. R. McMahon. 1991. Acid-base and ionic regulation, during and following emersion, in the freshwater bivalve, *Anodonta grandis simpsoniana* (Bivalvia: Unionidae). *Biol. Bull.* **181:** 289–297.

Byrne, R. A., B. N. Shipman, N. J. Smatresk, T. H. Dietz, and R. F. McMahon. 1991. Acid-base balance during prolonged emergence in the freshwater bivalve, *Corbicula fluminea*. *Physiol. Zool.* **64:** 748–766.

Deaton, L. E., and M. J. Greenberg. 1991. The adaptation of bivalve molluscs to oligohaline and freshwater: phylogentic and physiological aspects. *Malacol. Rev.* **24:** 1–18.

Deaton, L. E., J. G. S. Derby, N. Subhedar, and M. J. Greenberg. 1989. Osmoregulation and salinity tolerance in two species of bivalve mollusc: *Limnoperna fortunei* and *Mytilopsis leucophacta*. *J. Exp. Mar. Biol. Ecol.* **133:** 67–79.

Dietz, T. H. 1979. Uptake of sodium and chloride by freshwater mussels. *Can. J. Zool.* **57:** 156–160.

Dietz, T. H., and R. A. Byrne. 1990. Potassium and rubidium uptake in freshwater mussels. *J. Exp. Biol.* **150:** 395–405.

Finney, D. J. 1991. *Probit Analysis.* Cambridge Univ. Press, Cambridge.

Fisher, S. W., P. Stromberg, K. A., Bruner, and L. D. Boulet. 1991. Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorpha*: toxicity and mode of action. *Aquatic Toxicol.* **20:** 219–234.

Flatman, P. W. 1991. Mechanisms of magnesium transport. *Ann. Rev. Physiol.* **53:** 259–271.

Fyhn, H. J., and J. D. Costlow. 1975. Anaerobic sampling of body fluids in bivalve molluscs. *Comp. Biochem. Physiol.* **52A:** 265–268.

Graves, S. Y., and T. H. Dietz. 1982. Cyclic AMP stimulation and prostaglandin inhibition of Na transport in freshwater mussels. *Comp. Biochem. Physiol.* **71A:** 65–70.

Horohov, J., H. Silverman, J. W. Lynn, and T. H. Dietz. 1992. Ion transport in the freshwater zebra mussel, *Dreissena polymorpha*. *Biol. Bull.* **183:** 297–303.

Krogh, A. 1939. *Osmotic Regulation in Aquatic Animals.* Cambridge University Press, Cambridge.

McCorkle, S., and T. H. Dietz. 1980. Sodium transport in the freshwater Asiatic clam, *Corbicula fluminea*. *Biol. Bull.* **159:** 325–336.

McMahon, R. F. 1991. Mollusca: Bivalvia. Pp. 315–399 in *Ecology and Classification of North American Freshwater Invertebrates*, J. H. Thorp and A. P. Covich, eds. Academic Press, San Diego.

Murphy, W. A., and T. H. Dietz. 1976. The effects of salt depletion on blood and tissue ion concentrations in the freshwater mussel, *Ligumia subrostrata* (Say). *J. Comp. Physiol.* **108:** 233–242.

Nichols, S. J. 1993. Maintenance of the zebra mussel (*Dreissena polymorpha*) under laboratory conditions. Pp. 733–747 in *Zebra Mussels, Biology, Impacts and Control*, T. F. Nalepa and D. W. Schloesser, eds. Lewis, London.

Ram, J. L., and J. U. Walker. 1993. Effects of deionized water on viability of the zebra mussel, *Dreissena polymorpha*. *Comp. Biochem. Physiol.* **105C:** 409–414.

Ramcharan, C. W., D. K. Padilla, and S. I. Dodson. 1992. Models to predict occurrence and density of the zebra mussel, *Dreissena polymorpha*. *Can. J. Fish. Aquat. Sci.* **49:** 2611–2620.

Scheide, J. I., and T. H. Dietz. 1982. The effects of independent sodium and chloride depletion on ion balance in freshwater mussels. *Can. J. Zool.* **60:** 1676–1682.

Sprung, M. 1987. Ecological requirements of developing *Dreissena polymorpha* eggs. *Arch. Hydrobiol.* **79:** 69–86.

van der Velden, J. A., Z. I. Kolar, G. Flik, P. Polak, J. J. M. de Goeij, and S. E. Wendelaar Bonga. 1991. Magnesium transport between carp and water studied with $^{28}Mg^{2+}$ as radiotracer. *Appl. Radiat. Isot.* **42:** 347–352.

Vinogradov, G. A., N. F. Smirnova, V. A. Sokolov, and A. A. Bruznitsky. 1993. Influence of chemical composition of the water on the mollusk *Dreissena polymorpha*. Pp. 273–293 in *Zebra Mussels, Biology, Impacts and Control*, T. F. Nalepa and D. W. Schloesser, eds. Lewis, London.

Withers, P. C. 1992. *Comparative Animal Physiology.* Saunders, New York.